2611-Pos Board B41
Interactions between Pairs of Charges Buried in the Hydrophobic Interior of a Protein are Unexpectedly Weak
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Internal ion pairs buried in the hydrophobic protein interior are essential for many important biochemical processes, including H+ transport, e- transfer, ion homeostasis, and catalysis. Despite the importance of these pairs, their properties remain poorly understood. It has been suggested that, for some systems, medium or long-range Coulomb interactions between buried groups could play a role in determining biological function. In principle, this should only be possible if the protein interior behaves like a medium of low dielectric constant, as assumed in most electrostatics models. These motifs are then of special interest not only for their functional roles, but also as probes to examine the balance between Coulomb and hydration energies experienced by buried charges. As the distance between the internal charges increases, the balance of these energies will disfavor burying the groups in the charged state, eliminating any Coulomb interaction between them. To examine the balance between Coulomb and dehydration energies in the protein interior we engineered a series of double variants in a highly stable variant of staphylococcal nuclease. Each variant included an internal histidine paired with either an aspartate or glutamate buried at various internal positions throughout the protein interior. This set of proteins used to probe the distance dependence of potential Coulomb interactions. No significant electrostatic coupling was observed for pairs that interacted through medium- or long-range Coulomb interactions. When the distance between the groups was short, favorable coupling energies were only observed when the side chains of the pair could achieve a geometry favorable for a H-bond; spatial proximity alone was insufficient to create favorable Coulomb interactions. Simple electrostatics models that describe ionized states as point charges interacting through space are unlikely to be able to reproduce these data.

2612-Pos Board B42
A Reason for Long Tales
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Transcription Activator-Like Effectors (TALEs) are bacterial virulence factors containing a domain of repeats that recognize specific DNA sequences and reprogram transcription activation of invaded plant cells. TALE genes encode 5 to 30 repeats with average pairwise repeat identities greater than 91%. Most variability arises from only two positions termed Repeat Variable Diresidues (RVDs), which confer DNA binding specificity. Crystal structures of free and DNA-bound TALEs (Deng et al. *Science* 2012) show a large conformational change upon DNA binding. Thus, DNA binding is likely coupled to the free energy of tertiary structural change between the TALE repeats. We are interested in quantifying this relationship and relating it to folding cooperativity using nearest-neighbor (''Ising'') models.
To investigate the length dependence of folding, we created a set of consensus TALE constructs of varying length. Solubilizing N- and C-terminal caps are needed to favor monomeric protein in solution as detected by sedimentation velocity analytical ultracentrifugation. Capped consensus TALE repeat constructs have alpha-helical secondary structure as measured by farUV CD. Urea-induced unfolding transitions of TALE repeat arrays were measured and show cooperative unfolding transitions as well as increases in stability with length. These data are well-fitted by an Ising model, which separates the contributions of intrinsic and interfacial free energies. TALE repeats have an unfavorable intrinsic folding free energy of 5.3 kcal/mol and a favorable interfacial free energy of −6.8 kcal/mol. Using these values, the length dependence of TALE stability can be modeled, and shows TALEs under 5 repeats to be unfolded. Using the Ising parameters, we find that partially folded states with a single repeat unfolded are energetically accessible. Population of these partially folded states may be important for DNA binding.

2613-Pos Board B43
Effect of Gamma Radiation on the Structural and Functional Integrity of IgG
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Plasma-originated commercial intravenous immunoglobulin, which is used for a variety of clinical purposes, has been studied to determine the effect of virus-inactivating doses of gamma irradiation on the structural and functional characteristics of the protein. A detailed analysis has been performed in response to a concern that the use of conventional gamma irradiation may damage biologically active proteins. The results demonstrate that although gamma irradiation of the IgG may have some impact on protein structure, the damage can be reduced or even prevented by appropriate irradiation conditions. At the virucidal dose of gamma irradiation (50 kGy) and a temperature of −80oC, the integrity of the polypeptide chain of immunoglobulin and the secondary structure of IgG can be completely protected, while conformational changes in tertiary structure are significantly minimized to a level that preserves functional activity. The irradiated IgG retains specific antigen-binding properties and Fc-binding activity, indicating that the conformational integrity of the most important structural regions is not affected by gamma-irradiation. These results present strong evidence that gamma irradiation treatment can be effectively implemented for inactivation of pathogens in IgG solutions that are used for intravenous injection.

2614-Pos Board B44
Photoacoustic Calorimetry Studies of Ferric Cytochrome-C Folding using an No Photo-Trigger
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In this study, NO is utilized as a photo-trigger together with photoacoustic calorimetry to probe the kinetics, enthalpy and molar volume changes associated with the earliest folding events in ferric Cytochrome-c (Cc3+). The ferric heme protein was examined under different denaturing conditions including guanidine hydrochloride (2.8M GdnHCl) and Sodium dodecyl sulfate (SDS 0.4mM) (both in 50 mM Hepes buffer, pH ~7.5) along with NO resulting in the disruption of the axial heme Methionine-80 heme bond, triggering the partial unfolding of the complex. Under these conditions photo-dissociation of NO leaves the protein in a conformational state that favors refolding of the protein. The PAC data reveals three kinetic phases taking place subsequent to photolysis regardless of the denaturant environment. Specifically, in the presence of 2.8 M GdnHCl photolysis gave rise to kinetic events with lifetimes of <20 ns, ~860 ns, and ~6 μs that were associated with $\Delta H/\Delta V = -25$ kcal mol-1/-9 mL mol-1, 26 kcal mol-1/15mL mol-1, and 9 kcal mol-1/-26 mL mol-1, respectively. In the presence of 0.4 mM SDS, kinetic events were observed with lifetimes of <20 ns, ~640 ns, and ~8 μs with corresponding $\Delta H/\Delta V$ of −11 kcal mol-1/-1 mL mol-1, 5 kcal mol-1/3 mL mol-1, and 30 kcal mol-1/-9 mL mol-1, respectively. The data suggests a uniform mechanism for the early folding events occurring in the folding of Cc3+ complex subsequent to NO photo-dissociation which are attributed to NO dissociation from the heme, followed by reorganization of the distal pocket (i.e hydrogen bond formation/breakage, NO solvation, etc.) and potentially a intermolecular binding of Methionine (80 or 65) or Histidine (23 or 33) to the heme iron upon folding.

2615-Pos Board B45
Simulation of Pressure-Induced and Temperature-Induced Denaturation of Phosphoglycerate Kinase
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Phosphoglycerate kinase (PGK) is a 415-residue protein composed of two domains of almost equal size. Under the heat-induced denaturation, folding and unfolding of PGK is a two-state process. However, folding and unfolding of PGK under pressure-induced denaturation,is a three-state process. How the temperature and pressure affect the denaturation of PGK is still unknown. We aim to provide a molecular explanation for thermal and pressure denaturation by using coarse-grained molecular simulations where the interactions of residues experience expulsion of water molecules.

2616-Pos Board B46
Intermolecular Interactions in Highly Concentrated Protein Solutions Upon Compression and the Role of the Solvent
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The influence of high hydrostatic pressure on the structure and protein-protein interaction potential of highly concentrated lysozyme solutions up to about 370 mg mL^{-1} was studied and analyzed using small-angle X-ray scattering (SAXS) in combination with a liquid-state theoretical approach. In the concentration region below 200 mg mL^{-1}, the interaction parameters of lysozyme solutions are affected by pressure in a nonlinear way, which is probably due to significant changes in the structural properties of bulk water, i.e., due to a solvent-mediated effect. Conversely, for higher concentrated protein solutions, where

hydration layers below ~4 water molecules are reached, the interaction potential turns rather insensitive to compression. The onset of transient (dynamic) clustering is envisaged in this concentration range. Our results also show that pressure suppresses protein nucleation, aggregation and finally crystallization in supersaturated condensed protein solutions. These findings are of importance for controlling and fine-tuning protein crystallization. Moreover, these results are also important for understanding the high stability of highly concentrated protein solutions (as they occur intracellularly) in organisms thriving under hydrostatic pressure conditions such as in the deep sea, where pressures up to the kbar-level are reached.

2617-Pos Board B47

Atomistic and Coarse-Grained MD Simulations of the Intrinsically Disordered Bacillus Subtilis Ribonuclease P Protein

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The Bacillus subtilis Ribonuclease-P (RNase P) holoenzyme is a protein-RNA complex; however, the protein becomes disordered in the absence of its RNA binding partner. To identify the main determinants of why the RNase P protein becomes disordered, we performed (i) a statistical analysis of its interactions to identify the critical minimally frustrated residues that are important for structural stabilization, (ii) atomistic MD simulations in the presence and absence of the osmolyte Trimethylamine N-Oxide (TMAO), which has been shown experimentally to stabilize its native structure even without its RNA binding partner, and (iii) coarse-grained Go-type MD simulations to determine the folding/unfolding mechanism. We observed an intermediate in our coarse-grained MD simulations that we propose is the partially disordered state in the absence of its RNA binding partner and the intermediate seen experimentally in TMAO-induced folding experiments. We also identified critical residues for stabilization that can be tested experimentally using standard mutagenesis folding kinetic experiments.

2618-Pos Board B48

The Dock-and-Coalesce Mechanism for the Association of Intrinsically Disordered WASP with the Cdc42 GTPase

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Intrinsically disordered proteins (IDPs) play key roles in signaling and regulation. Many IDPs undergo folding upon binding to their targets. We have proposed that coupled folding and binding of IDPs generally follow the dock-and-coalesce mechanism, whereby a segment of the IDP through diffusion docks to its cognate subsite and subsequently the remaining segments coalesce around their subsites [PCCP 14:10466(2012)]. Here we tested the validity of this mechanism on the association between the intrinsically disordered GTPase binding domain (GBD) of the Wiskott-Aldrich Syndrome protein (WASP) and the Cdc42 GTPase, by both experiment and computation. The association rate constants (ka) were measured by stop-flow fluorescence under various solvent conditions and temperatures. ka reaches 107 M-1s-1 at physiological ionic strength and has a strong salt dependence, suggesting that an electrostatically enhanced, diffusion-controlled docking step is rate-limiting. Diffusion control is supported by an inversely proportional relation between ka and the solvent viscosity with glucose as the viscogen. ka increases with increasing temperature; though the increase is larger than expected from the effect of temperature on the protein diffusion constants, the discrepancy may be accounted for by the effect of temperature in decreasing the solvent dielectric constant, leading to stronger electrostatic rate enhancement. Similarly, a modest decrease in ka by urea may be rationalized by the latter's effect in increasing the solvent dielectric constant. Our computation, based on the transient-complex theory [Structure 19:1744(2011)], identified the N-terminal basic region of the GBD as the docking segment, which has strong electrostatic complementarity with the cognate subsite. Our study suggests that the dock-and-coalesce mechanism allows WASP and other IDPs to code electrostatic complementarity into the docking segment to gain binding speed and use additional interactions formed by the coalescing segments to reinforce binding affinity.

2619-Pos Board B49

Cooperative Helix Formation in the (AAQAA)3 Peptide Obtained with the Drude Polarizable Force Field

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Molecular simulations can provide atomic-level details of protein folding. However, their accuracy is limited by approximations made in the underlying empirical force fields. Recently we presented a force field for peptides and proteins that includes explicit treatment of electronic polarization based on the classical Drude oscillator model.[1] The Drude force field was found to maintain protein native structures during microsecond molecular dynamics simulations of multiple folded proteins, and leads to significant variability of backbone and side chain dipole moments as a function of environment.[2] Here we report replica exchange simulations of the helix-forming $(AAQAA)_3$ peptide and the β-sheet-forming GB1 hairpin using this fully polarizable model.

Polarizable simulations of $(AAQAA)_3$ reveal the presence of folding cooperativity consistent with experimental observations. The cooperativity is significantly larger than that modeled by currently available non-polarizable force fields and is shown to be directly associated with enhanced dipole moments of the peptide backbone upon helix formation.[3] The GB1 hairpin is found to be less stable with the Drude force field compared to the experimental observation. Results from these extensive condensed phase simulations of peptide folding will be utilized, together with QM calculations of model alanine polypeptide systems, to further refine the backbone parameters in the Drude protein force field. In summary, our results demonstrate that the inclusion of explicit electronic polarizability leads to a fundamentally improved model of the physical forces dictating the structure and dynamics of polypeptides.

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2620-Pos Board B50

Dependence of Internal Friction on Native Topology

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Apparent internal friction has been identified in the folding rates of several proteins and in the dynamics of unfolded proteins, via their dependence on solvent viscosity. Theoretical and computational studies have attributed this phenomenon, at least in part, to local barrier crossing events, in particular torsion angle isomerizations. However, there are still many systems for which no internal friction has been observed in experiment, and others for which the effect is only weak. Here, we elucidate the origin of this discrepancy by studying a model system whose native state can be either an α-helix or a β-hairpin. Remarkably, we find clear apparent internal friction for the helix, but no evidence of it for the hairpin. These results are consistent with the observation that proteins whose folding rates exhibit internal friction tend to be α-helical. We can explain the difference by examining the occurrence of torsional transitions along the folding coordinate of the hairpin and helix. Finally, we consider the folding of the Trp cage mini protein, whose structure includes both helical and hairpin elements. We obtain two barriers – the major barrier for formation of the hairpin and a minor barrier for initial helix formation, allowing us to dissect the contribution of internal friction to different parts of the energy landscape. We find a clear signature of internal friction for the helical barrier and, within the uncertainty of our calculation, little internal friction for the hairpin barrier. Our results show how variation of solvent viscosity can be used to probe features of folding mechanisms.

2621-Pos Board B51

Thermodynamics of β-Structures from Molecular Dynamics Simulations

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β-Sheets are some of the most common secondary structure motifs in proteins, and are important for mediating protein-protein interactions through their association. This association can also lead to the aggregation of misfolded proteins into β-pleated-sheets in neurodegenerative disorders like amyloidosis. The folding pathway from random coil to β-sheet usually involves two competing process: (1) the collapse of a hydrophobic core, and (2) the formation of intrapeptide hydrogen bonds. It has been proposed, and shown computationally, that the hydrophobic core collapse precedes hydrogen bond formation. In this study we examine the thermodynamics of β-hairpin formation for the GB1 domain of protein G with molecular dynamics simulations by calculating a two-dimensional free energy surface in both vacuum and explicit water using as our reaction coordinates (1) the radius of gyration of the hydrophobic core and (2) the number of native hydrogen bonds, corresponding to the two aforementioned folding processes, respectively. We also compare the results of different versions of the CHARMM force field, namely CHARMM22, CHARMM22/CMAP, CHARMM22* and CHARMM36. Finally, we show how these methods can be applied to other β-structures in vivo, namely β-helix structures in the outer membrane of Gram-negative bacteria.